#1162 A Nectin-4 Targeted TLR9 Agonist Antibody Conjugate Induces Robust Immune Cell Activation and Anti-Tumor Responses

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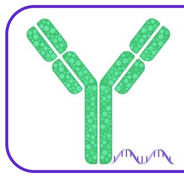
Introduction

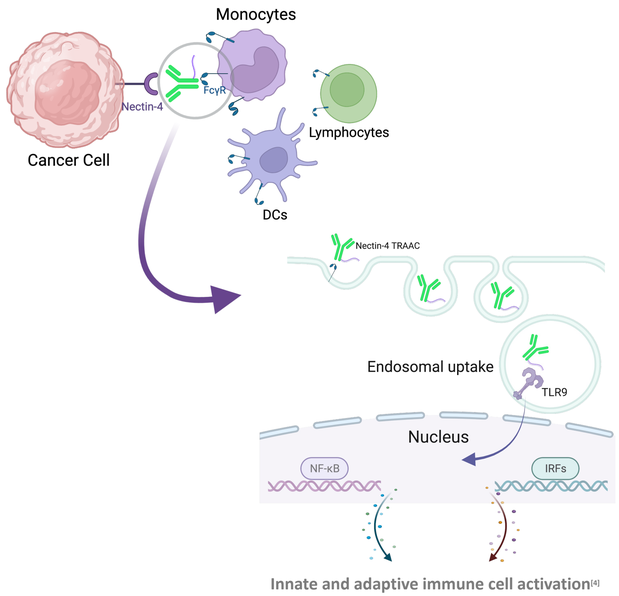
- Novel therapies engaging both innate and adaptive immune response may produce more robust and durable anti-cancer immunity [1].
- Activation of Toll-Like Receptor 9 (TLR9) by unmethylated CpG oligodeoxynucleotides (CpG-ODNs) promotes innate inflammatory responses and the induction of adaptive immunity [1][2]. Several intratumorally injected CpG-ODNs demonstrated clinical responses in melanoma patients [3].
- We developed a Toll-like Receptor Agonist Antibody Conjugate (TRAAC) comprised of an optimized CpG-ODN (T-CpG) conjugated to a novel Nectin-4-targeting antibody for systemic administration that enables delivery of a potent TLR9 agonist to the tumor microenvironment (TME).
- Nectin-4 is a cancer-associated antigen over-expressed in many solid tumors with limited expression in normal tissues. Additionally, Nectin-4 over-expression correlates with poor prognosis [2].
- We present preclinical data demonstrating that Nectin-4 TRAAC triggers TLR9 signaling, induces myeloid and dendritic cell activation, cancer cell phagocytosis, pro-inflammatory cytokine production and lymphocyte activation, altogether resulting in potent anti-tumor efficacy.

Fig. 1: Nectin-4 TRAAC: a Toll-like Receptor Agonist Antibody Conjugate designed for systemic and tumor-targeted delivery of T-CpG, an optimized TLR9 agonist



Antibody: anti-Nectin-4
- Localizes to TME of Nectin-4 expressing tumors
- ADCC & ADCP through engagement of Fcγ receptors

T-CpG
- Proprietary TLR9 agonist
- Optimized for potency and stability
- Efficient site-specific conjugation



Experimental Results

Fig. 2: Anti-Nectin-4 antibody displays species cross-reactivity and selective binding to Nectin-4

A

Antibody	K_D(nM)			
	Human Nectin-4	Mouse Nectin-4	Cynomolgus monkey Nectin-4	Human Nectin-1
anti-Nectin-4	5	16	6	NB

B

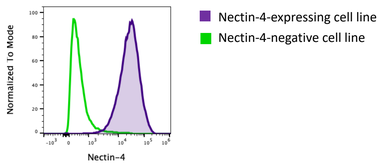


Figure 2: (A) Results of SPR 1:1 binding kinetics fitting using extracellular domains of human Nectin-4, mouse Nectin-4, cynomolgus monkey Nectin-4, and human Nectin-1 proteins. NB = no binding. (B) Results from flow cytometry showing that the novel Nectin-4-targeting antibody binds to a Nectin-4-expressing cancer cell line, whereas no binding is detected on a non-expressing cancer cell line. No binding to human PBMCs was observed in the same assay (data not shown).

Fig. 3: Nectin-4 TRAAC activates dendritic, myeloid and B cells in human PBMCs co-cultured with Nectin-4+ cancer cells

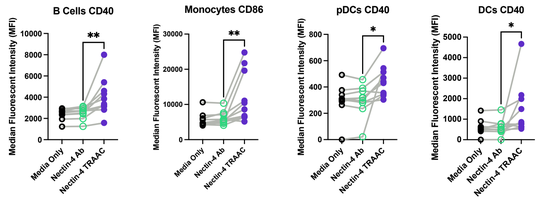


Figure 3: Human PBMCs from 10 healthy donors were co-cultured with Nectin-4 expressing cancer cells and were left untreated (media only) or treated with unconjugated Nectin-4 antibody or Nectin-4 TRAAC. After 24-hour incubation, the expression levels of cell surface markers indicative of cell activation were determined by flow cytometry, * p<0.05, ** p<0.006 (one-way ANOVA).

Fig. 4: Nectin-4 TRAAC induces monocyte- and macrophage- mediated phagocytosis of Nectin-4+ cancer cells

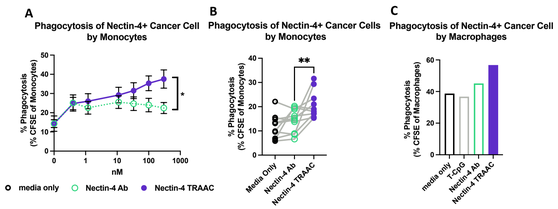


Figure 4: Phagocytosis of cancer cells as assayed by flow cytometry. (A) Human PBMCs were co-cultured with CFSE labeled Nectin-4 expressing cancer cells and incubated with titrated unconjugated Nectin-4 antibody or with Nectin-4 TRAAC for 24 hours. Phagocytic monocytes were identified as CFSE+CD14+ cells. (B) Human PBMCs from 10 healthy donors were co-cultured with CFSE labeled Nectin-4 expressing cancer cells and incubated with media only, unconjugated Nectin-4 antibody or Nectin-4 TRAAC for 24 hours. Phagocytic monocytes were identified as CFSE+CD14+ cells. (C) Monocyte-derived macrophages were co-cultured with CFSE labeled Nectin-4 expressing cancer cells and incubated with unconjugated Nectin-4 antibody, Nectin-4 TRAAC or T-CpG alone for 2 hours. Phagocytic macrophages were identified as CFSE+CD33+ cells, * p< 0.05, ** p<0.006 (paired t-test and one-way ANOVA respectively).

Fig. 5: Nectin-4 TRAAC triggers TLR9 signaling and elicits pro-inflammatory cytokine production in human PBMCs co-cultured with Nectin-4+ cancer cells

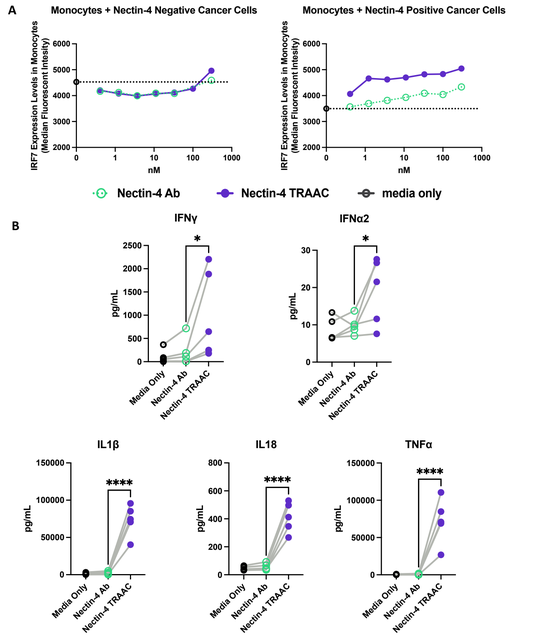


Figure 5: (A) Human PBMCs were co-cultured with Nectin-4 expressing and non-expressing cancer cells and left untreated or incubated with titrated unconjugated Nectin-4 antibody or Nectin-4 TRAAC for 24 hours. IRF7 expression in monocytes was determined by flow cytometry. (B) Human PBMCs from 5 healthy donors were co-cultured with Nectin-4 expressing cancer cells and were left untreated or incubated with unconjugated Nectin-4 antibody or Nectin-4 TRAAC for 24 hours. Cytokine levels were determined by cytokine bead array (flow cytometry), * p<0.04, **** p<0.0001 (one-way ANOVA).

Fig. 6: Nectin-4 TRAAC monotherapy exhibits potent efficacy in multiple syngeneic tumor models including checkpoint inhibitor (CPI) refractory model, EMT6

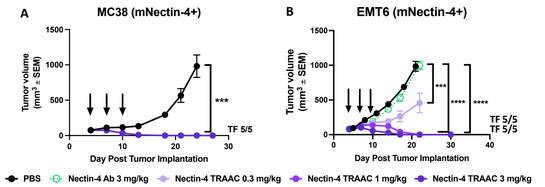


Figure 6: (A) Immune competent mice bearing subcutaneous MC38 expressing murine Nectin-4 were dosed intraperitoneally (i.p.) with PBS or Nectin-4 antibody conjugated to murine reactive T-CpG (mT-CpG). *** p = 0.0002 (unpaired t-test) (B) immune competent mice bearing subcutaneous EMT6 expressing murine Nectin-4 were dosed i.p. with PBS, unconjugated Nectin-4 antibody, or various doses of Nectin-4 antibody conjugated to mT-CpG. Arrows indicate doses administered. *** p = 0.0004, **** p<0.0001 (one-way ANOVA). TF = tumor-free

Fig. 7: Nectin-4 TRAAC monotherapy exhibits efficacy in a tumor model with low Nectin-4 expression levels

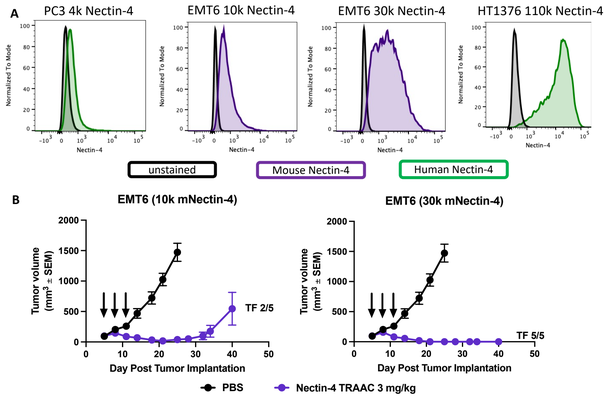


Figure 7: (A) Nectin-4 expression levels and average copy number in EMT6 cells engineered to express various levels of murine Nectin-4 in comparison to levels of Nectin-4 endogenous expression in human cancer cells. Results are from flow cytometric analysis using species cross-reactive antibody. (B) Mice (n=5/group) bearing EMT6 tumors expressing murine Nectin-4 as shown in (B) were dosed i.p. with PBS, or Nectin-4 antibody conjugated to mT-CpG. Arrows indicate doses administered, TF = Tumor-free.

Fig. 8: Nectin-4 TRAAC monotherapy results in durable responses and anti-tumor immunological memory

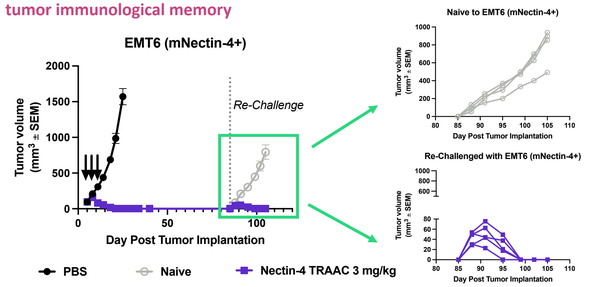


Figure 8: Tumor-eradicated mice from EMT6 over-expressing murine Nectin-4 following 3 mg/kg 3q3 systemic treatment (arrows) with Nectin-4 antibody conjugated to mT-CpG were re-challenged ~70 days post initial tumor clearance. Tumor and treatment naïve, age-matched, mice were used as a control for tumor growth.

Summary and Conclusions

- We generated high-affinity, species cross-reactive, antibodies specifically targeting Nectin-4. The Nectin-4 antibodies were conjugated with our optimized TLR9 agonist to generate Nectin-4 TRAAC.
- Nectin-4 TRAAC targets both innate and adaptative immune cells via Fcγ receptor engagement leading to robust pro-inflammatory activity in cancer cell-PBMC co-cultures.
- Nectin-4 TRAAC promotes phagocytosis of Nectin-4-expressing cancer cells.
- Systemic administration of single agent Nectin-4 antibody conjugated to mT-CpG produces curative efficacy in multiple Nectin-4-expressing syngeneic tumor models, including those refractory to checkpoint inhibitors and tumors with low Nectin-4 expression levels, and triggers anti-tumor immunological memory.
- The preclinical data of Nectin-4 TRAAC supports its development for solid tumor malignancies that express Nectin-4.

References:
1. Hamid, O., Ismail, R. and Puzanov I., Intratumoral Immunotherapy-Update 2019. Oncologist, 2020 Mar;25(3):e423-e438.
2. Chatterjee S., Sinha S., Kundu CN. Nectin cell adhesion molecule-4 (NECTIN-4): a potential target for cancer therapy. Eur J Pharmacol. 2021 Nov 15;911:174516.
3. Liu, M., O'Connor, R., Trefely, S. Graham, K., Snyder NW., Beatty, GL. Metabolic rewiring of macrophages by CpG potentiates clearance of cancer cells and overcomes tumor-expressed CD47-mediated 'don't eat me signal". Nat Immunol. 2019 Mar; 20(3):265-275.
4. Created with BioRender.com